

Application Note

Optimizing Plasmid Yield via AmMag™ Quatro Maxi-1400 Using Thomson Ultra Yield® Plasmid System

Janet Caverly¹, Nilojan Jehanathan¹, Rudy Zhao¹, Luciana Rosselli-Murai¹

Alberto Estevez², Brian Janssen², Sam Ellis²

¹Automated Protein & Plasmid Purification Technology Lab, GENSCRIPT USA INC, 20 Kingsbridge Rd., Piscataway, NJ, 08854, USA

²Thomson Instrument Company, 2091 Rutherford Road Suite 150, Carlsbad, CA 92008, USA

Introduction

The AmMag™ Quatro Maxi-1400 provides a fully automated user-friendly solution to high quality maxi-scale plasmid purification from *Escherichia coli* cultures. This system features a controller (GenScript, Cat#D00018) that can connect to up to 4 independent modules (GenScript, Cat#D00019), enabling users to process up to 24 samples in a highly customizable, yet hands-free high throughput manner.

The system comes with an all-inclusive kit containing a reusable waste bin and 24 sets of each of the following: tip boxes, prefilled reagent cartridges, reaction tubes and sample tubes. The prefilled reagent cartridges enable purification of up to 1.50g bacterial pellets – although users are able to purify a diverse range of pellet sizes from 0.50 to 1.50g. With the all-inclusive kit, users may expect to reach up to 1mg yield for high-copy plasmids.

With optimal growth conditions, users typically need 40-100mL for TB (Terrific Broth), 100-200mL for 2xYT (2x Yeast Extract Tryptone), and 200-300mL of LB (Luria-Bertani) Media to attain the recommended bacterial pellet size of 1.30 to 1.50g for optimal yield and concentration, subsequently subjecting users to multiple time-consuming centrifugation cycles for proper pellet collection in the 50mL AmMag™ Quatro Maxi-1400's sample tubes.

Thomson Plasmid+® Enriched Media (Thomson Instrument Company, Cat#446300) is an optimized microbial growth medium formulated for enhanced plasmid production and *E. coli* cell density. Plasmid+® Enriched Media allows users to use less media to attain a 1.50g bacterial pellet, removing the need for multiple centrifugation cycles, resultingly reducing time in the overall plasmid purification process via AmMag™ Quatro Maxi-1400 in addition to improved concentration and yield.

In this application note, we evaluate how Plasmid+® Enriched Media in combination with Ultra Yield Flasks (Thomson Instrument Company, Cat#931147) and Vent Caps (Thomson Instrument Company Cat#899109) can be implemented into the AmMag™ Quatro Maxi-1400 workflow to maximize efficiency and produce plasmids suitable for downstream applications including but not limited to transfection, cloning, and sequencing. In Part One, we compare the performance of bacterial pellets grown in Plasmid+® and TB media by analyzing concentration, purity and endotoxin levels. In Part Two, we perform a titration of binding bead volumes on pellets harvested from Plasmid+® media to identify optimal protocols for plasmid purification in addition to assessing the potential effects of binding beads on overall purity and quality of plasmids.

Material and Methods

Transformation

The pcDNA3.1(+) plasmid (GenScript) containing an ampicillin resistance gene was transformed into Top10 competent *E. coli* cells according to the manufacturer's protocol (Invitrogen, Cat#C404003). The transformed cells were plated on an LB agar plate with 100 µg/mL ampicillin and incubated at 37°C for 16 h.

Bacterial Culture

A single colony was picked from the plate and inoculated into 4mL starter culture containing either Plasmid+® or TB media. The culture was grown at 37°C with shaking speed at 220 rpm for 8 hours in a 25 mm orbital shaker (Eppendorf Innova® 40, Cat#M1299-0090).

Part 1

Starter culture was inoculated at 1:1000 into its respective culture medium with Antifoam 204 (Sigma, Cat#A8311) diluted to manufacturer's protocol, containing 100 µg/mL ampicillin. The resulting culture was distributed in triplicates into 125mL Ultra Yield® Flasks with varying volumes of 25, 30, 35 and 40mL. The flasks were capped with Ultra Yield® 125mL Vent Cap (Thomson Instrument Company, Cat#899109) and incubated at 37°C with shaking at 350 rpm for 16h in a 25 mm orbital shaker (Eppendorf Innova® 40, Cat#M1299-0090).

Part 2

Plasmid+® starter culture was inoculated at 1:1000 into 24 x 30mL of Plasmid+® consisting of Antifoam 204, diluted according to manufacturer's protocol, and 100µg/mL ampicillin in 125mL Ultra Yield® Flasks capped with Ultra Yield® 125mL Vent Caps. The flasks were incubated at 37°C with shaking speed at 350RPM in 25mm orbital shaker. 12 flasks were reserved for 24h cultivation while the other remaining flasks were reserved for 16h

cultivation.

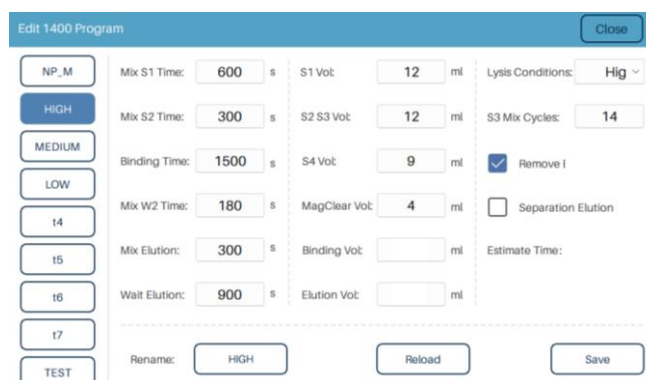
Pellet preparation

To harvest the cells, each flask's culture was spun down into one 50 mL Quatro sample collection tube. The tubes were centrifuged at 4,000 × g for 10 minutes at 4°C. After centrifugation, the cell pellets were weighed and recorded before being frozen and stored at –20°C for further use.

DNA Purification

Purification of the pcDNA3.1(+) plasmid was performed using the AmMag™ Quatro 1400 with the standard kit (GenScript, Cat#L00882-24), following "High" protocol method (Figure 1), according to the instrument manual. The final collection samples were spun down at 7000 x g for 15 minutes at room temperature for the final elution step.

A

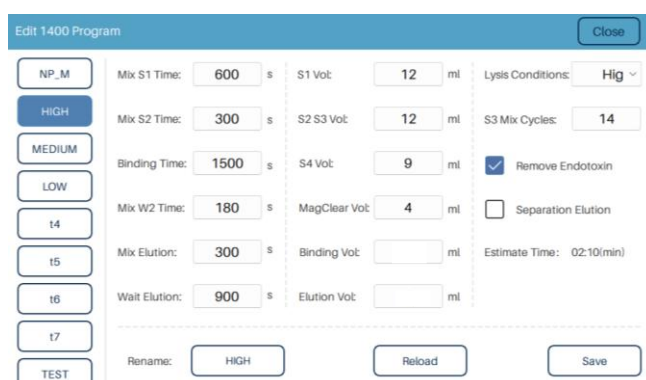


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NP_M	Mix S1 Time: 600 s	S1 Volt: 12 ml	Lysis Conditions: Hig
HIGH	Mix S2 Time: 300 s	S2 S3 Volt: 12 ml	S3 Mix Cycles: 14
MEDIUM	Binding Time: 1500 s	S4 Volt: 9 ml	☒ Remove I
LOW	Mix W2 Time: 180 s	MagClear Volt: 4 ml	☐ Separation Elution
t4	Mix Elution: 300 s	Binding Volt: ml	Estimate Time:
t5	Wait Elution: 900 s	Elution Volt: ml	
t6			
t7			
TEST			

Rename: HIGH [Reload] [Save]

B



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NP_M	Mix S1 Time: 600 s	S1 Volt: 12 ml	Lysis Conditions: Hig
HIGH	Mix S2 Time: 300 s	S2 S3 Volt: 12 ml	S3 Mix Cycles: 14
MEDIUM	Binding Time: 1500 s	S4 Volt: 9 ml	☒ Remove Endotoxin
LOW	Mix W2 Time: 180 s	MagClear Volt: 4 ml	☐ Separation Elution
t4	Mix Elution: 300 s	Binding Volt: ml	Estimate Time: 02:10(min)
t5	Wait Elution: 900 s	Elution Volt: ml	
t6			
t7			
TEST			

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Figure 1 Quatro Protocols Used to Purify Plasmid DNA from Bacterial Pellets (A) Part 1 Protocol used to purify plasmids from bacterial pellets grown in varying volumes of Plasmid+® or TB media: 25, 30, 35 and 40mL (B) Part 2 Protocol used to purify bacterial pellets with varying binding beads/elution

buffer volumes: 1.5/1.0, 2.0/1.5, 2.5/2.0 and 3.0/2.5. Elution buffer volume was increased alongside binding beads to allow for maximized plasmid recovery.

Concentration, Purity and Analysis

The concentration and purity of pcDNA3.1(+) plasmid samples were determined using a NanoPhotometer® N50 (IMPLEN, Cat#N50-Touch). Two µL of sample was loaded on the pedestal and the absorbance at 230, 260, and 280 nm was measured.

Endotoxin levels were measured using the Endosafe® nexgen-PTS™ (Charles River Laboratories Cat#PTS150K). Samples were diluted 1:100 mL/mL in LAL reagent water (GenScript) and loaded into Endosafe® LAL cartridges (Charles River Laboratories, Cat#PTS5505F).

Quality assurance was conducted using supercoil analysis. The resulting samples were loaded onto a 1.0% agarose gel, electrophoresed at 150 V for 30 minutes, and visualized under UV light. Supercoil analysis was performed using Image Lab software (Bio-Rad).

Results and Discussion

Part 1

Comparing Plasmid Yield in Bacterial Pellets Harvested from Plasmid+® and TB Media

To compare the performances of samples cultivated from Plasmid+® and TB media, the bacterial pellets' weights were recorded (Figure 2). Four conditions were tested by varying the volume of culture used (25, 30, 35 and 40mL). To reach optimal pellet weight 1.50g, users may only need 30mL of Plasmid+® compared to 40mL of TB media, requiring 25% less media using the same growth conditions. On average, Plasmid+® delivered ~26% more bacterial pellet.

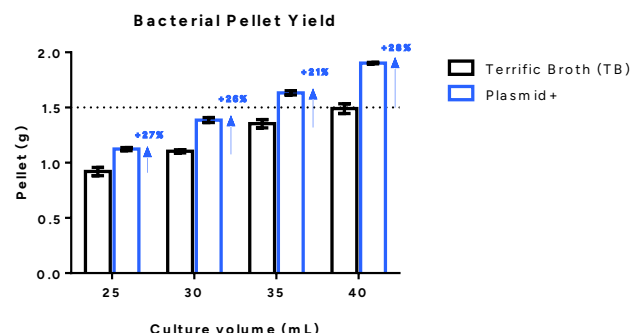


Figure 2 Comparing Bacterial Pellet Yield from TB Media (Black) versus Plasmid+® Enriched Media (Blue) Cultures

Bar graph representing differences in bacterial yield with percentages representing increased pellet weight between medias.

Results via nanophotometer demonstrated consistently improved plasmid concentration and yield in samples grown from Plasmid+® media compared to TB media (Figure 3). Most notably in 25mL culture volume, Plasmid+® demonstrated higher concentration and yield than TB media with up to 73% and 60% increase, respectively. As TB media increased in increments of 5mL, concentration and yield increased on average by 14% and 10%, respectively. Conversely, concentrations remained consistent as Plasmid+® culture volume increased in 5mL increments, averaging 1090.08ng/µL across all 12 samples. This potential occurrence due to oversaturated binding beads was further evaluated in Part Two.

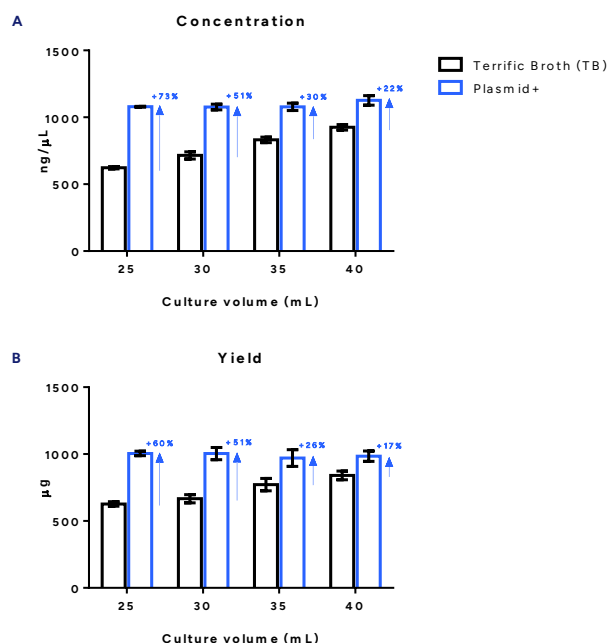


Figure 3 Concentration and Yield in Bacterial Pellets Grown in TB (Black) and Plasmid+® (Blue) (A) Bar graph demonstrating difference in performance of both medias for plasmid concentration represented by percentages. (B) Bar graph demonstrating the difference in performance of both medias for plasmid yield represented by percentages.

Comparing Purity, Endotoxin Levels and Supercoil Ratio

Evaluation of the effects of different culture media on purity and structural integrity of the plasmids was performed.

Purity measurements including A260/280 and A260/230 ratios were optimal amongst all plasmid samples ranging from 1.8 to 2.0 and above 2.0, respectively indicating minimal protein and/or guanidine salt contamination (Figure 4). The consistent ratios indiscriminately across all plasmid samples suggested minimal to negligible effects of Plasmid+® media on plasmid purity. Therefore, spectrophotometric analysis of the pcDNA3.1(+) cultivated from both medias showed comparable results for purity.

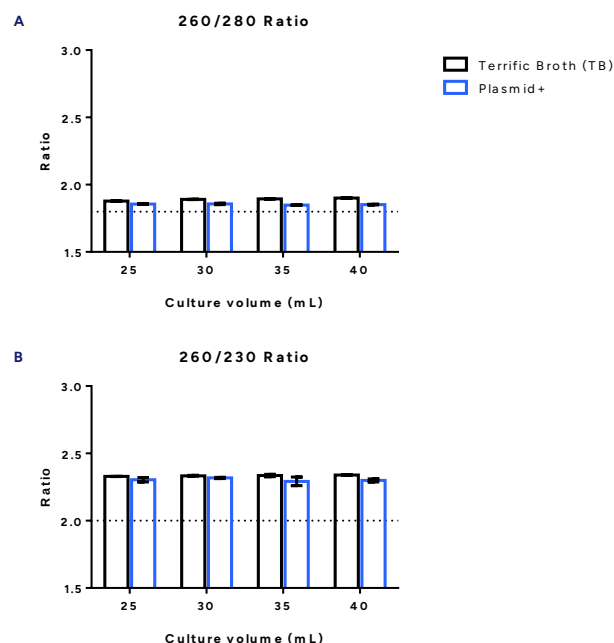


Figure 4 Absorbance at 230, 260 and 280nm of Plasmids from Bacterial Pellets Cultivated from TB (Black) and Plasmid+® (Blue) (A) Graph representing A260/280 ratios of pcDNA3.1(+) samples cultivated from TB and Plasmid+® with the acceptable range of 1.80-2.00. (B) Graph representing A260/230 ratios of pcDNA3.1(+) samples cultivated from TB and Plasmid+® with the acceptable range at >2.0.

Residual endotoxin levels were measured for both sets of plasmids to compare the effects of the culture conditions on endotoxin. Samples cultivated from Plasmid+® ranged from <5.00 to 22 EU/mL or <0.004 to 0.019 EU/μg (Figure 5). These values were consistent with the endotoxin levels of the samples cultivated in TB media. TB samples ranged from <5.00 to 22.6 EU/mL or <0.007 to 0.039 EU/μg. Such results support that the Plasmid+® media did not negatively affect the final endotoxin levels of the plasmid samples. Endotoxin analysis of the pcDNA3.1(+) plasmids cultivated from both medias showcase comparable performance using GenScript standard kit and protocol.

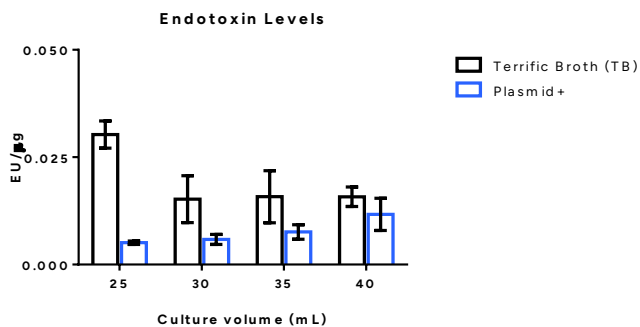


Figure 5 Endotoxin Levels Analysis in Purified Plasmids from Bacterial Pellets Cultivated from TB (Black) and Plasmid+® (Blue) Graph displaying endotoxin levels in EU/μg demonstrated plasmids well below transfection grade of 0.1 EU/μg for both sets of samples.

The structural integrity of the purified plasmids was verified by agarose gel electrophoresis. All samples visualized at a supercoil ratio of at least 90% with negligible genomic DNA contamination thus supported comparable performance of both medias (Figure 6).

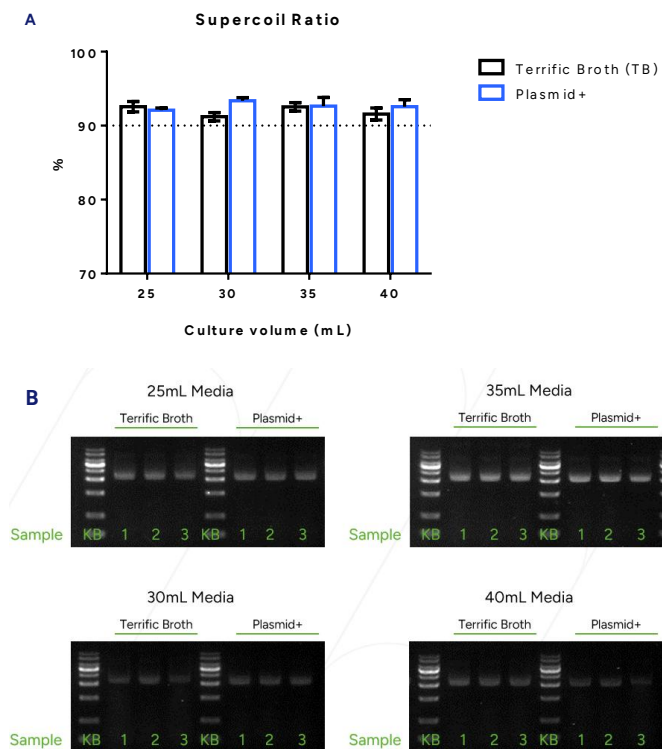


Figure 6 Comparing Supercoil Ratio of pcDNA3.1(+) Samples (A) Bar Graph representing the supercoil ratios of pcDNA3.1(+) cultivated from Plasmid+® (Blue) and TB (Black)

media with >90% marked as the acceptable range of plasmid quality. (B) Gel analysis of plasmid supercoil ratio visualized via agarose gel.

As such, users may implement Plasmid+® into their workflow for pellet preparation as final plasmid samples demonstrated a comparable, and in some cases, an enhanced, performance compared to TB media with maintained suitability for sensitive downstream applications.

Part 2

Optimizing Plasmid Yield with Plasmid+® and AmMag™ Quatro Maxi-1400

Bacterial pellets from 30mL cultures were harvested after 16h and 24h cultivation in Plasmid+® and recorded for pellet weight. Consistent pellet weights throughout all samples demonstrate that cultivation time provided minimal to no effects on final bacterial pellet weight (Figure 7). Both cultivation times yielded pellets within optimal recommended pellet mass of approximately 1.50g for purification via AmMag™ Quatro Maxi-1400, further demonstrating the consistency and reliability of Plasmid+ performance including when implemented into the AmMag™ Quatro Maxi-1400 workflow.

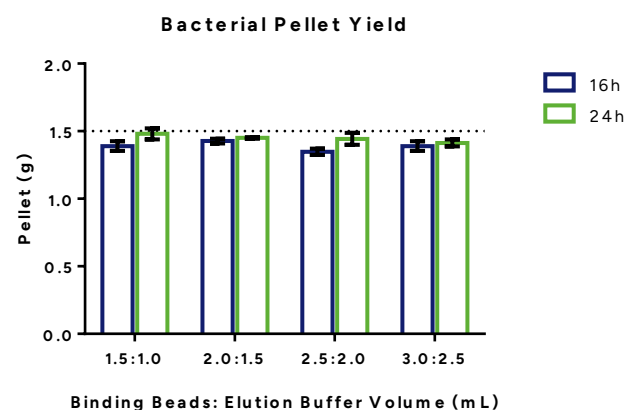


Figure 7 Bacterial Pellet Yield of Samples Cultivated in Plasmid+® for 16h (Dark Blue) and 24h (Green) X axis demonstrates the subsequent titration of binding beads used to maximize plasmid yield.

Results via nanophotometer showed similar trends

in plasmids from 16h and 24h cultivation. There was a gradual increase in plasmid yield as binding beads and elution volume increased from 1.5mL and 1.0mL to 2.5mL and 2.0mL, respectively with a plateau after 2.5mL binding beads. Expectantly, concentration of the pcDNA3.1(+) samples decreased as binding beads and elution buffer volume increased (Figure 8). The plateau in yield (~2mg of plasmid) after 2.5mL binding beads volume suggested optimal yield can be attained from protocols with 2.5mL/2.0mL binding beads and elution buffer volume, respectively. In summary, cultivation in Plasmid+ medium resulted in a plasmid DNA yield of approximately 2 mg from a 30 mL culture, whereas an equivalent 30 mL TB culture yielded approximately 0.67 mg. These results indicate that the Plasmid+ medium increased plasmid production by more than twofold under the tested conditions.

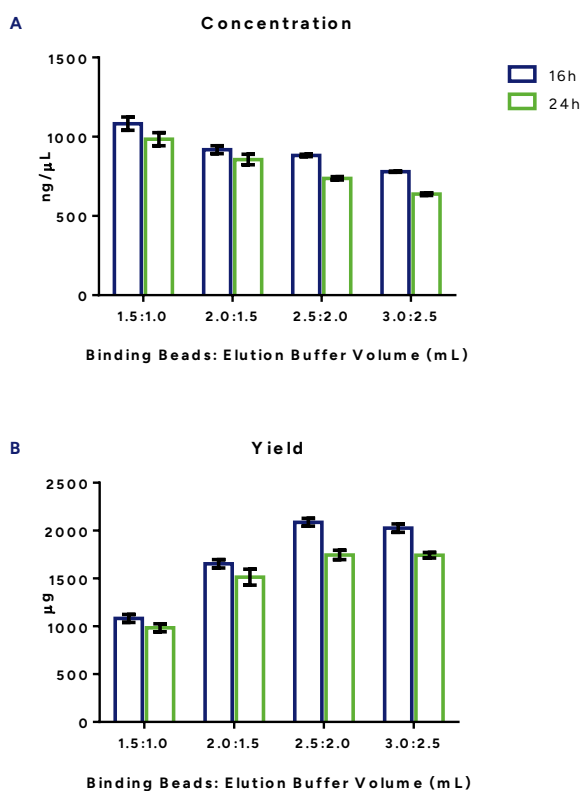


Figure 8 Plasmid Concentration and Yield Comparing Bacterial Pellets Grown in Plasmid+® for 16h (Dark Blue) and 24h (Green) (A) Bar graph representing concentration

of plasmids resulting from binding beads: elution volume titration (B) Yield of plasmids resulting from binding beads: elution buffer titration.

Visual analysis of preclear beads post purification implicated higher frequency of incomplete lysis occurred in bacterial pellets harvested after 24h cultivation compared to 16h cultivation (Figure 9). The incomplete lysis further verified the observed slight decrease in concentration and yield for 24h samples.

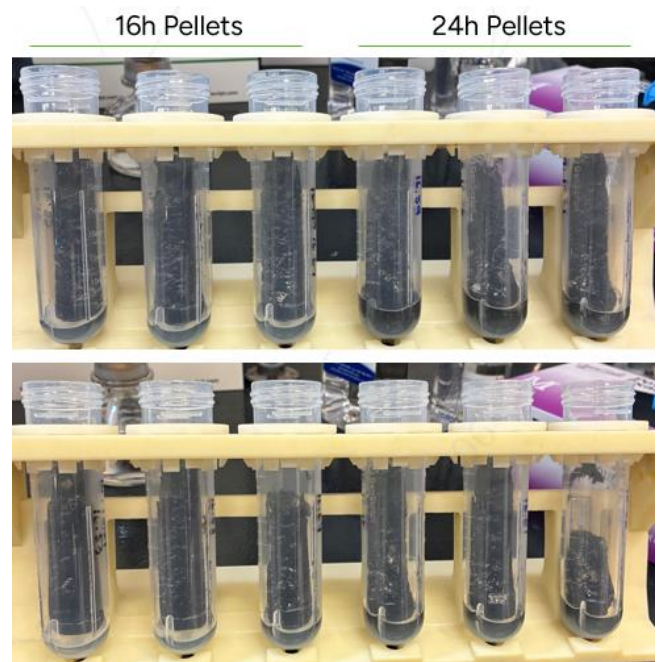


Figure 9 Side by Side Comparison of Preclear Beads Post Purification Comparing 16h and 24h Pellets Incomplete lysis occurred at higher frequency in 24h pellets than 16h pellets as observed in white precipitate leftover in preclear beads for 24h pellets despite high lysis conditions.

Bacterial pellets harvested at 16h and purified at 2.5mL binding beads and 2.0mL elution buffer volume demonstrated optimal yield.

Comparing Purity and Supercoil Ratio

Evaluation of the effects of varying binding beads volume on plasmid purity and supercoil ratio was assessed.

Purity measurements including A260/280 and A260/230 ratios were optimal amongst all plasmid samples ranging from 1.8 to 2.0 and above 2.0,

respectively indicating minimal protein and/or guanidine salt contamination (Figure 10). The consistent ratios indiscriminately across all plasmid samples suggested minimal to negligible effects of varying binding beads and elution buffer volume. Therefore, spectrophotometric analysis of the pcDNA3.1(+) samples showed comparable results for purity.

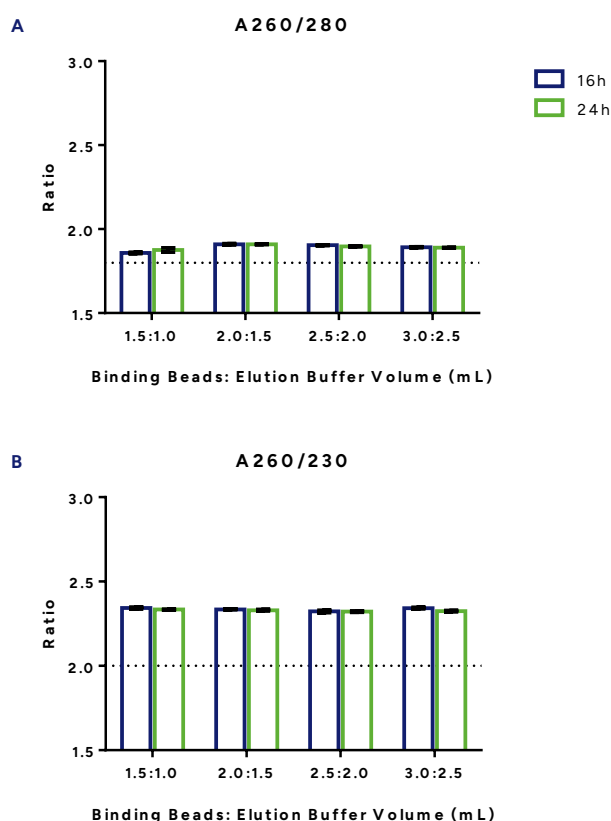


Figure 10 Purity of Plasmids Assessed via Absorbance at 230, 260 and 280nm (A) Graph representing A260/280 ratios of pcDNA3.1(+) samples cultivated from Plasmid+® with the acceptable range of 1.80-2.00. (B) Graph representing A260/230 ratios of pcDNA3.1(+) samples cultivated from Plasmid+® with the acceptable range at >2.0.

The structural integrity of the purified plasmids was verified by agarose gel electrophoresis. All samples from 16h cultivation regardless of binding bead volume demonstrated a consistent supercoil ratio of at least 90% (Figure 11). Noticeably, increasing growth conditions to 24h caused a

significant decrease in supercoiling, albeit still predominantly supercoiled (Figure 11). This demonstrated that growth at 16h produced optimal and consistent plasmid supercoiling.

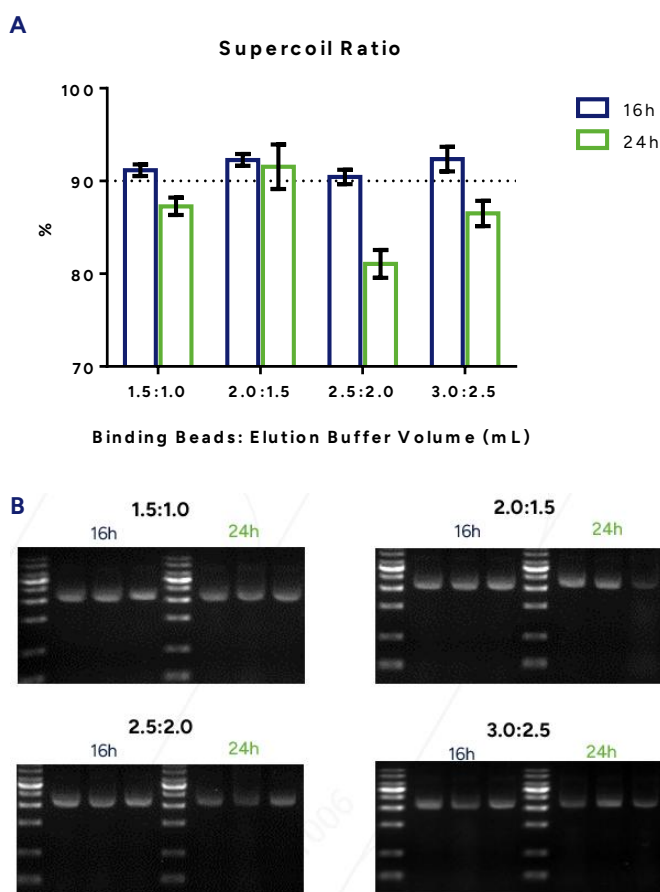


Figure 11 Supercoil Ratio Analysis of pcDNA3.1 Samples After Binding Bead Titration (A) Bar Graph representing the supercoil ratios of pcDNA3.1(+) cultivated from Plasmid+® for 16h (Dark Blue) and 24h (Green) with >90% marked. (B) Gel analysis of plasmid supercoil ratio visualized via agarose gel.

While concentration and yield were affected by varied binding bead and elution buffer volumes, purity and supercoiling of plasmids were not affected at 16 hours samples.

Incomplete lysis is a common cause of low yield and poor plasmid purity in maxi preps. Incomplete lysis leaves bacterial debris, nucleases, and chromosomal DNA in the lysate, which can reduce yield, contaminate the plasmid, and cause downstream issues such as poor transfection

efficiency, DNA degradation and poor supercoil levels.

Conclusion

Implementing Thomson Ultra Yield Plasmid System: Ultra Yield® Flask, Plasmid+® Media and Vent Cap into the AmMag™ Quatro Maxi-1400 workflow removed the necessity for multiple pellet centrifugation cycles.

With only 30mL of Plasmid+® media needed for an optimal bacterial pellet, users will be required to use less media for enhanced plasmid concentration and yield without harming quality, purity and endotoxin level.

Together, the Thomson Ultra Yield® Plasmid System: Ultra Yield® Flasks, Plasmid+® and Vent Cap and AmMag™ Quatro Maxi 1400 system provide a fully automated solution to plasmid purification and time efficient pellet preparation step, making maxi preparations seamless, consistent and straight forward.